UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY AND DIAGNOSIS OF HUMAN LEPTOSPIROSIS IN MALAYSIA

ISAM MOHAMED ALI MOHAMED EL-JALII

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By

ISAM MOHAMED ALI MOHAMED EL-JALII

Thesis Submitted in Fulfilment of the Requirements for the Degree of
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September 2000

DEDICATION

TO THE MEMORY OF MY MOTHER, FATHER AND NEPHEW KHATAB

TO MY WIFE EMTENAN AND DAUGHTER RAYAN

TO MY BROTHERS AND SISTERS

TO ALL OF THEM WITH LOVE AND GRATITUDE

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: Prof. Dr. Abdul Rani Bahaman

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Retrospective study of human leptospirosis in Malaysia based on microscopic agglutination test (MAT) showed 13% overall prevalence of infection for the period 1983-1998. Results indicated that the prevalence was decreasing in the last five years (1994-1998). The prevalence was highest among Indians (16.67%) followed by Malays (11.48%) and the least among Chinese (5.88%). The 20-29 year-old group showed the highest prevalence of infection (17.13%). Less than 10 year-old group showed the least prevalence of infection (5.66%). Generally, many of the cases occurred between the ages of 20-50 years. Serological survey based on enzyme-linked immunosorbent assay (ELISA) showed a high overall prevalence (12.56%) of leptospiral infection. Kuala Lumpur showed the highest prevalence (19%) whilst Penang recorded the lowest prevalence (6.67%). No significant differences in the prevalence between the other states was noted.

A comparative study of three serological tests, namely enzyme-linked immunosorbent assay (ELISA), microscopic agglutination test (MAT) and indirect hemagglutination (IHA), test was carried out to evaluate these tests in the diagnosis of human leptospirosis. A total of 3000 serum samples from three groups of people were examined. In Group I, IgM and IgG-ELISA were able to detect a number of cases in the first sampling before MAT titres were detectable. In the second sampling, all samples positive for MAT were also positive for IgM-ELISA. IHA test gave positive reactions with only 38% of the samples while all samples were positive for ELISA. In Group II, ELISA detected IgM and IgG to leptospires in the samples which were negative to MAT. These were samples from patients with clinical signs of leptospirosis.

The polymerase chain reaction (PCR) was evaluated as a tool for diagnosis of leptospirosis and differentiation of leptospiral strains. Urine samples with as little as 10 serovar *hardjo* cells per ml of urine were positive on PCR indicating high sensitivity of the test. Detection of small number of leptospiral cells in urine by PCR was an advantage over culture. Random amplification polymorphic DNA (RAPD) fingerprinting was applied to differentiate leptospiral strains. Two primers were tested for their abilities to generate individual RAPD fingerprints. The DNA profiles obtained with each primer were distinct and reproducible. The fingerprint obtained could be useful for distinguishing the serovars up to the strain level. Profiles obtained revealed genetic heterogeneity between serovars belong to one serogroup.

Analysis of leptospiral DNA with restriction enzymes, *Hind* III, *BamH* I and *EcoR* I revealed a high heterogeneity between the serovars examined. This high heterogeneity may be due to the large genome of the genus *Leptospira.* No relationship was found between the restriction patterns and the species from which the isolate was isolated. Similarities were observed among isolates of the same serovar. The 10 leptospiral field isolates were assayed for presence of plasmid DNA. Only two isolates were found to harbour plasmid DNA. The plasmid profiling obtained is of limited epidemiological value for differentiation of leptospiral isolates.

It appears that human leptospirosis is an endemic infection in Malaysia. The findings showed that ELISA was a suitable serological test for diagnosis of leptospirosis compared to MAT and IHA tests. On the other hand, the application of PCR and REA in the diagnosis of leptospirosis would be useful.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EPIDEMIOLOGI DAN DIAGNOSIS LEPTOSPIROSIS DI MALAYSIA

Oleh

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Kajian retrospektif terhadap leptospirosis manusia di Malaysia berasaskan ujian pengaglutinatan mikroskopi (MAT) menunjukkan prevalens jangkitan keseluruhan 13% untuk tempoh 1983 - 1998. Hasil kajian menunjukkan prevalens ini semakin kurang dalam tempoh lima tahun terakhir (1994-1998). Prevalens ini paling tinggi di kalangan kaum India (16.67%) diikuti kaum Melayu (11.48%) dan paling sedikit di kalangan kaum Cina (5.88%). Kumpulan umur 20-29 menunjukkan prevalens jangkitan paling tinggi (17.13%). Kumpulun umur kurang daripada 10 tahun menunjuk prevalens paling rendah (5.66%). Umumnya, banyak daripada kes ini berlaku pada kumpulan umur 20 - 25 tahun. Tinjauan serologi berasaskan asai imunoserap terangkai enzim (ELISA) menunjukkan prevalens jangkitan leptospira keseluruhan tinggi (12.56%). Kuala Lumpur menunjukkan prevalens paling tinggi (19%), manakala Pulau Pinang merekodkan prevalens paling rendah (6.67%). Tiada kelainan tererti wujud di antara negeri lain.

Suatu kajian perbandingan terhadap tiga ujian serologi, iaitu asai imunoerap terangkai enzim (ELISA), ujian pengaglutinatan mikroskopi (MAT), dan ujian penghemaglutinatan tak langsung (IHA) telah dijalankan untuk menilai ujian ini dalam diagnosis leptospirosis manusia. Sejumlah 3000 sampel serum manusia telah diperiksa. Dalam kumpulan I, IgM- dan IgG-ELISA dapat mengesan beberapa kes dalam pensampelan pertama, sebelum titer MAT dapat dikesan. Dalam pensampelan kedua, kesemua sampel yang positif dengan MAT juga positif dengan IgM-ELISA. Ujian IHA memberikan tindak balas positif untuk 38% daripada sampel sahaja, sambil kesemua sampel positif dengan ELISA. Dalam kumpulan II, ELISA mengesan IgM dan IgG terhadap leptospira dalam sampel yang negatif dengan MAT. Ini ialah daripadsa pesakit yang menunjukkan petanda klinikal untuk leptospira.

Tindak balas berangkai polimerase (PCR) telah dinilai sebagai alat untuk diagnosis leptospirosis dan untuk pembezaan strain leptospira. Sampel urin yang mengandungi hanya 10 *hardjo* sel per ml urin masih positif dalam ujian PCR, menunjukkan tingginya kepekaan ujian tersebut. Pengesan sejumlah sel yang begitu kecil dalam urin melalui PCR memberi ujian ini kelebihan berbanding pengkulturan. Penyidikjarian DNA polimorfus penguatan rawak (RAPD) telah diguna untuk membezakan strain leptospira. Dua primer telah diuji untuk keupayaannya menjanakan sidikjari RAPD individu. Profil DNA yang diperolehi daripada setiap primer ini adalah nyata dan boleh dihasil semula. Sidikjari yang diperolehi mungkin

berguna untuk membeza serovar sehingga pada aras strain. Profil diperolehi menunjukkan keheterogenan genetik di antara serovar daripada satu serokumpulan.

Analisis DNA leptospira dengan enzim pengehadan, *Hind* III, *BamH* I dan *Ecor*R I menunjukkan keheterogenan tinggi di antara serovar yang diperiksa. Keheterogenan tinggi ini mungkin disebabkan oleh genom besar pada genus *Leptospira*. Tiada perkaitan yang ditemui di antara pola pengehadan dan spesies daripada mana pencilan itu diperolehi. Persamaan telah dicerap di kalangan pencilan yang serovarnya sama. Pencilan 10 leptospira liar telah diasaikan untuk kewujudan DNA plasmid. Hanya dua pencilan telah ditemui mengandungi DNA plasmid. Pemprofilan plasmid yang diperolehi itu mempunyai nilai epidemiologi terhad dalam pembezaan pencilan leptospira.

Nampaknya leptospirosis manusia itu adalah suatu jangkitan endemik di Malaysia. Penemuan ini menunjukkan ELISA itu merupakan ujian serologi yang sesuai untuk diagnosis leptospirosis berbanding ujian MAT dan IHA. Disebaliknya, penggunaan PCR dan REA dalam diagnosis leptospirosis adalah memuaskan.

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I certify that an Examination Committee met on 22nd September 2000 to conduct the final examination of Isam Mohamed Ali Mohamed El-Jalii on his Doctor of Philosophy thesis entitled "Epidemiology and Diagnosis of Human Leptospirosis in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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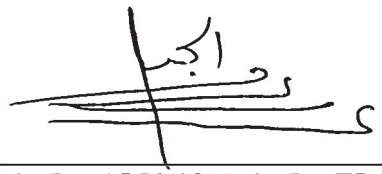
I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.



ISAM MOHAMED ALI MOHAMED EL-JALII

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LIST OF ABBREVIATIONS

Ab	Antibody
ABTS	2,2-azino-bis (3-ethylbenzthiazoline-6-Sulfonic acid)
AP-PCR	Arbitrary primed polymerase chain reaction
Bp	Base pair
BSA	Bovine serum albumin
°C	Degree Celcius
CAAT	Cross agglutination absorption test
CFT	Complement fixation test
CHEF	Contour-clamped homogenous electric field
CSF	Cerobrospinal fluid
D.D	De-inonized distilled
dNTP	Deoxy-nucleotide triphosphate
DFM	Dark-field microscopy
DNA	Deoxyribonucleic acid
Dr.	Doctor
EDTA	Ethylene diamine tetra-acetone
ELISA	Enzume-linked Immunosorbent assay
FAO	Food and Agriculture Organization
FAT	Fluorescent antibody test
g	Gram
g/l	Gram/litre
G+C	Guanine+Cytisine
H_2O_2	Hydrogen peroxide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect haemagglutination
IMR	Institute for Medical Research
JS	Johnson and Seiter
Kbp	Kilobase pairs
K L	Kuala Lumpur
L	Leptospira
LPS	Lipopolysaccharide
M	Molar
MAT	Microscopic agglutinaton test
Mda	Megadalton
mM	Milimole
M.S	Molecular size
M.W	Molecular weight
N.S	Negri Sembilan
O.D	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

pH	Hydrogen-ion concentration
PLGE	Pulse field gel electrophoresis
PUO	Pyrexia of unknown origin
RAPD	Random amplified polymorphic DNA
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
rpm	Round per minute
SAT	Slide agglutination test
SDS	Sodium dodecyl sulphate
U	Unit
UK	United Kingdom
UM	Universiti Malaya
UPM	Universiti Putra Malaysia
US	United States
USA	United States of America
UV	Ultraviolet
µg	Microgram
µl	Microlitre
µM	Micro mole
V	Volt
VRI	Veterinary Research Institute
V/V	Volume/volume
WHO	World Health Organization
%	Percent

CHAPTER I

INTRODUCTION

Leptospirosis, also known as "march fever" and "mud fever," is an important zoonotic disease, with important veterinary and public health impact (Dikken and Kmety, 1978; Gussenhoven *et al.*, 1997; Marcos *et al.*, 1997). Leptospirosis is caused by *Leptospira interrogans.* Based on immunological tests, more than 200 leptospiral seorvars have been identified. The serovars could be placed into 23 serogroups (Soltys, 1979; Woodward *et al.*, 1997; Chu *et al.*, 1998). In Malaysia, 38 serovars from 13 serogroups have been known to occur (Bahaman, 1988).

In livestock, the disease causes important economic losses. Although this disease is usually mild and often subclinical, it can lead to great losses due to abortions, stillbirths, infertility, mastitis, weak progeny, decreased milk production, and with certain leptospiral serovars, death (Songer *et al.*, 1983; Thiermann, 1984; Bey and Johnson, 1986).

Leptospiral serovars can infect mammals including man. However, the pathogenicity and clinical manifestations of the disease depend on the animal